Evaluation of our results shows the yield of polysaccharide production has been increased from 1.6 mg/L of fermentation broth which is reported by other investigators to 4.9 mg/L. As it has been found in sepharose 4-B Cl column chromatography and a HPLC profile, vi-CPS retained its native molecular weight and configurational stability. Besides, the results obtained from an agarose-gel immune diffusion technique and serum bacteriocidal assay revealed that vi-CPS retained its immunogenic properties as well. Concerning the observation of the results obtained from this investigation, the downstream process of production and purification of vi-CPS adopted in the Pasteur Institute of Iran is a reliable technique to yield large-scale polysaccharide typhoid vaccine.

Summary #67

Hb D PUNJAB [BETA 121 (GH4) GLU: GLN] IN WESTERN IRAN: SINGLE HETEROZYGOUS, HOMOZYGOUS AND COMPOUND HETEROZYGOUS WITH BETA-THALASSEMIA

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While individuals carrying Hb D-Punjab are typically asymptomatic and even those homozygous for this hemoglobin variant have a relatively benign condition, diagnosis could be important for genetic counseling. We have studied clinical and hematological features of Hb D-Punjab in Western Iran. Hematological indices of 22 subjects from 11 unrelated families living in Western Iran (Kermanshah, Ilam and Kurdistan provinces) exhibiting Hb D were measured by an automated H1 blood counter, DAEA-52 column chromatography and electrophoresis. The Hb D-Punjab status of all individuals was confirmed by PCR followed by digestion with *Eco*RI. 19 out of 22 individuals that were simple Hb D-heterozygotes had, on average, 39.2% Hb D and 0.89% Hb F. All were asymptomatic with normal hematological indices. A 37-year-old woman was found to be homozygous for Hb D-Punjab (91% Hb D-Punjab), but free of clinical symptoms with normal hematological indices. Two patients were double heterozygous for Hb D-Punjab and β-thalassemia. Hematological indices of these two patients with Hb D-β-thalassemia showed the presence of hypochromia and microcytosis along with elevation of Hb F (12% and 18%). The present study confirms the benign nature of homozygous Hb D-Punjab and indicates compound heterozygosity for Hb D-Punjab and β-thalassemia confers a thalassemic-minor syndrome to individuals carrying this combined hemoglobinopathy.

Summary #68

CONFORMATIONAL LOCK AND THERMAL INACTIVATION KINETICS OF EUPHORBIA AMINE OXIDASE

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The kinetics of thermal inactivation of copper-containing amine oxidase from euphorbia latex (ELAO) were studied in a 100-mM sodium phosphate buffer, pH 7, using cadavarine as the substrate. The thermal inactivation curves were not linear at 60°C and 64°C; three linear phases were shown. The first phase gave some information about the number of dimeric forms of the enzyme that were induced by the higher temperatures using the "conformational lock" pertaining theory to oligomeric enzyme. Based on the "conformational lock" theory we estimated that there should be three contact sites between two subunits of ELAO. Since the 3D structure of ELAO has not been determined, we used the X-ray structure of Pea Seedling amine oxidase (PSAO) for determining the contact sites. Alignment of the amino acid sequences of ELAO and PSAO showed that these contact sites are conserved, which are mostly located at the C-terminal region of amine oxidases. The contact sites are located very near the active site of the enzyme so that it is predictable that destruction of the contact site can lead to inactivation of the enzyme, i.e., the dimeric form is necessary for amine oxidase activity. The second and third phases were interpreted according to a dissociative thermal inactivation model and rate constants of dissociation and denaturation were determined.

Summary #69

THE EFFECT OF A CROWDING AGENT ON THE CHAPERONE ACTION OF ALPHA-CRYSTALLIN

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The small heat-shock protein (sHsp), alpha-crystallin, acts as a molecular chaperone stabilizing proteins under stress conditions through the formation of a soluble sHsp-substrate complex. In this study, the effects of alpha-crystallin during its interaction with a variety of substrate proteins (ovotransferrin, insulin, alpha-lactalbumin, Beta low-crystallin) in the presence and in the absence of a macromolecular crowding agent were examined using visible absorption spectroscopy, tryptophan fluorescence spectroscopy, ANS binding assay, circular dichroism, NMR, HPLC and TEM. Using dextran as a macromolecular crowding agent, the rate and extent of aggregation of reduced ovotransferrin, insulin, alpha-lactalbumin and Beta low-crystallin have been altered. Under these conditions, alpha-crystallin was found less effective in preventing aggregation and precipitation of target proteins. It is proposed that a kinetic competition exists between aggregation of a stressed protein and the chaperone action of alpha-crystallin. The presence of dextran leads to a rapid aggregation and precipitation of ovotransferrin, a-lactalbumin and Beta low-crystallin upon reduction and heating which supports the hypothesis that alpha-crystallin interacts more effectively with slowly aggregating rather than rapidly aggregating target proteins. However, alpha-crystallin had approximately the same effect on aggregation of insulin both in the presence and in the absence of dextran.

Summary #70

DIVERSE ROLES RUN FOR ACTIVE CENTER OF RNASE A

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The kinetics of RNase A versus RNA as a natural substrate or cytidine 2′,3′-cyclic phosphate (cCMP) as a nucleotide substrate obey a non-hyperbolic mechanism (J. Protein Chem 2000; 19:335–345) because of the existence of intermediate transitions among the RNase catalytic process. This was confirmed by a differential scanning calorimetry (DSC) profile of the RNase-3′CMP (cytidine 3′-monophosphate as the end product of hydrolysis of cCMP) complexes which show a splitting of two distinct DSC peaks with different structural stabilities. The bifurcate appearance of the DSC profile manifests a physical view of a light kinetic structural transition via complete enzymatic hydrolysis of cCMP. The direct binding (non-enzymatic reaction) of RNase A with 3′CMP indicates a single DSC profile and monophasic binding isotherm (Thermochimica Acta 2004; 411:37–42). Here, the structural and functional roles of His119 and His12 in the active center of RNase A were studied by H NMR, DSC, fluorescence, circular dichroism and pH gradient techniques via diethylpyrocabonate (DEPC) modification. Carbethoxylation of His119 was followed by conversion of A conformation to B form in the active site and for His12 carbethoxylation was accompanied by a second spatial rotation of the corresponding imidazole ring in the active site to adopt a new conformation (J. Protein Chem 2003; 22:643–654). Also, the distance between Ns1–His119 and Ne2–His12 was determined upon addition of sulfate ions as kosmotropic salts and thiocyanate ion as a chaotropic salt by a molecular dynamic method. The results indicate the compactness (measured by microviscometry) and decrease of the catalytic center of RNase A in the presence of sulfate ions. This was concomitant with enzyme activation. In contrast, expansion of the enzyme surface and elongation of the catalytic center caused by the addition of thiocyanate ion